



S-93,994

Attachment 1

**Marked up version of the specification
showing the change made**

IN THE SPECIFICATION:

Please amend the specification as follows:

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1. On page 23, the 1st full paragraph starting on line 13 should read as follows:

The homozygous ixr1-1 -- (SEQ ID NO. 1) -- and ixr1-2 -- (SEQ ID NO. 2) -- mutant plants are 300 and 90 times respectively, more resistant to isoxaben than wild type plants (Heim et al. 1989). ixr1-1 (ixr1-2 was not tested) is also more resistant to a new thiazolidinone herbicide (TZ, compound 1). At 12µM the thiazolidinone herbicide kills wild type Arabidopsis, but reduces growth of the ixr1-1 mutant by only 50% (Sharples et al., 1998)

2. On page 17, the 1st paragraph starting on line 1 should read as follows:

oligonucleotide primers (F: 5 - - ' - - "=" CGAACTTGAGACCTCTTGA 3 - - ' - - "=" - -) - - -
(SEQ ID NO. 4) - - ; - - (- - R: 5 - - ' - - "=" GCTTACCTGGAGACAGTCA 3') - - (SEQ ID NO. 5) - -
were designed with the Oligo Primer Analysis Software version 5.0 (National Biosciences, Plymouth, MN) to PCR-amplify a 124 bp fragment from genomic wild-type Columbia DNA, containing the (TG)₁₃ dinucleotide repeat. Using the same primers a shorter PCR-product (<120bp) is obtained from wild-type Landsberg genomic DNA, whereas a Col x Ler heterozygous plant give both products, as can be easily seen on 4% agarose-gels. PCR-conditions for SSLP-marker med24.2: 50 mM KCL, 10mM Tris-HCL (pH 9.0 @ 25°C), 0.1% Triton X-100, 200µM dATP, dGTP, dTTP, dCTP (each), 5 pmoles primer F, 5 pmoles primer R, 2.0mM MgCl₂, 1.0 Units Taq. Polymerase (Promega, Madison, WI), 10-50 ng genomic DNA, final volume 20µl. PCR-program: 1 min 94°C; 40 cycles (20 sec 94°C 20 sec 55°C, 40 sec

72°C), 3 min 72°C.

3. On page 17, the 1st full paragraph starting on line 12 should read as follow:

SSLP-marker moj9.2 (approx. 100kb proximal of m217 on the physical map of chromosome V; http://www.kazusa.or.jp/arabi/chr5/pmap/P1_map_1.html)

molecular target: (TA)₁₉ dinucleotide repeat (bases 53618-53655) on chromosome V BAC-clone MOJ9

PCR primers: F: 5' - ' - - " = " CATGATCCATCGTCTTAGT 3' - ' - - " = " - - (SEQ ID NO. 6) - -

R: 5' - ' - - " = " AATATCGCTTGTTTTCGC 3' - - (SEQ ID NO. 7) - -

PCR-product size: 179 bp in Col, ca. 160b bp in Ler

PCR-conditions: as for med24.2 with 2.2mM MgCl₂

4. On page 18, the 1st full paragraph starting on line 5 should read as follows:

SSLP-marker muk11.1 (approx. 500 kb proximal of med24.2 on the physical map of chromosome V; http://www.kazusa.or.jp/arabi/chr5/pmap/P1_map_1.html)

molecular target: (GA)₃₈ dinucleotide repeat (bases 57187-57252) on chromosome V BAC-clone MUK11

PCR primers: F: 5' TCCAAAGCTAAATCGCTAT 3' - - (SEQ ID NO. 8) - -

R: 5' CTCCGTCTATTCAAGATGC 3' - - (SEQ ID NO. 9) - -

PCR-product size: 177 bp in Col, ca. 120b bp in Ler

PCR-conditions: as for med24.2.

5. On page 18, the 2nd full paragraph starting on line 12 should read as follows:

SSLP-marker nga158 (approx. 500kb distal of moj9.2 on the physical map of

chromosome V; http://www.kazusa.or.jp/arabi/chr5/pmap/P1_map_1.html)

molecular target: (CT)₁₄ dinucleotide repeat (bases 19384-19411) on BAC-clone MJJ3
(<http://www.kazusa.or.jp/arabi/chr5/map/0-2Mb.html>)

PCR primers: F: 5' ACCTGAACCATCCTCCGTC 3' -- (SEQ ID NO. 10) --
R: 5' TCATTTTGGCCGACTTAGC3' -- (SEQ ID NO. 11) --

PCR-product size: 108 bp in Col, ca. 104b bp in Ler, (http://genome-www3.stanford.edu/cgi-bin/Webdriver?Mlval=atdb_caps_max&oid=2f10.210e)

PCR-conditions: as for med24.2, except that the annealing temperature in the PRC was raised to 60°C

6. On page 19, the 1st full paragraph staring on line 1 should read as follows:

SSLP-marker ng225 (approx. 36 kb proximal of muk11.1 on the sequencing map of chromosome V; <http://www.kazusa.or.jp/arabi/chr5/map/0-2Mb.html>)

molecular target: imperfect (GA)₂₁ dinucleotide repeat (bases 12203-12244) on chromosome V BAC-clone MUG13

PCR primers: F: 5' TCTCCCCACTAGTTTTGTGTCC 3' -- (SEQ ID NO. 12) --
R: 5' GAAATCCAAATCCCAGAGAGG 3' -- (SEQ ID NO. 13) --

PCR-product size: 119 bp in Col, ca. 189 bp in Ler, (http://genome-www3.stanford.edu/cgi-bin/Webdriver?Mlval=atdb_caps_max&oid=2f10.2112)

PCR-conditions: as for med24.2, except that MgCl₂ 1.75 mM.

7. On page 19, the 2nd full paragraph should read as follows:

CAPS-marker MUG13E (approx. 60 kb proximal of nga225 on the right end of BAC-clone MUG13 on the sequencing map of chromosome V;
<http://www.kazusa.or.jp/arabi/chr5/map/0-2Mb.html>)

molecular target: Accl restriction enzyme site, which is present in the sequence of ecotype Landsberg erecta, but not Columbia.

PCR primers: F: 5' GATTTCCCCAGACGATTT 3' - - (SEQ ID NO. 14) - -
 R: 5' AGTTTATTTGTTGCGGTTTT 3' - - (SEQ ID NO. 15) - -

PCR-product size: 2033 bp fragment (bases 79,441-81,473 of BAC MUG13) in Col and Ler before Accl-digest. 1228 and 805 bp fragments in Ler after digest.

PCR-conditions: as for PAI1-marker, but annealing temperature was 54°C.

Accl-digest: 7µl of PCR-product were mixed with 9µl H₂O, 2µl 10x buffer M (Amersham), 2µl BSA (1mg/ml) and 1U Accl, and digested for 5 hrs at 37°C. 10µl of each digest were analyzed on a 1.2% agarose gel.

8. The paragraph starting on the 3rd paragraph of page 19 (line 24) and continuing to page 20 line 17 should read as follows:

CAPS-marker PAI2 (approx. 33 kb distal of nga158 on the physical map of chromosome

V; http://www.kazusa.or.jp/arabi/chr5/pmap/P1_map_1.html)

molecular target: AflIII restriction enzyme site, which is present in PAI2-gene from ecotype Columbia, but not Landsberg erecta.

PCR primers: F: 5' CAGTTAATGAAACAAGCTTTGTTC 3' - - (SEQ ID NO. 16) - -
 R: 5' GTTGAGAAAATCACTTTGGTG 3' - - (SEQ ID NO. 17) - -

PCR-product size: 645 bp fragment (bases 45928-46572 on BAC clone MOP10) in Col and Ler before AflIII-digest. 590 and 55 bp fragments in Col after digest.

PCR-conditions: 50 mM KCL, 10mM Tris-HCL (pH9.0 @ 25°C), 0.1% Triton X-100, 250 µM dATP, dGTP, dTTP, dCTP (each), 5 pmoles primer F, 5 pmoles primer R, 2.5 mM MgCl₂, 1.0 Units *Taq* Polymerase (Promega), 40-50 ng

genomic DNA, final volume 25 µl. PCR-program: 1 min 94°C; 35 cycles (20 sec 94°C, 20 sec 58°C, 90 sec 72°C), 3 min 72°C.

AFIII-digest: 7 µl of PCR-product were mixed with 9µl H₂O, 2 µl 10 x buffer H (Amersham), 2µl BSA (1mg/ml) and 1U AfIII, and digested for 5 hours at 37°C. 10 µl of each digest were analyzed on a 2.5% agarose gel.

9. On Page 20, the 2st full paragraph starting on line 18 should read as follows:

SSLP-marker k18i23.1 (approx. 101 kb proximal of PAI2 on the sequencing map of chromosome V; <http://www.kazusa.or.jp/arabi/chr5/map/0-2Mb.html>)

molecular target: purine-rich stretch (bases 17830-17870) on chromosome V BAC-clone K18I123. Length Polymorphism was detected by comparative sequencing of the Ler and Col genomic sequence of that region.

PCR primers: F: 5' TGGTTAGATTTGCTGTT 3' -- (SEQ ID NO. 18) --
R: 5' ATTCTGCATTATTAGTTGTC 3' -- (SEQ ID NO. 19) --

PCR-product size: 139 bp in Col, 133 bp in Ler

PCR-conditions: as for med24.2, except that MgCl₂ was 2.5 mM and the annealing temperature during PCR was lowered to 48°C.

10. On Page 21, the first full paragraph starting on line 14 should read as follows:

Genomic DNA for each genotype was prepared from a mixture of young growing leaves and inflorescence tissue using the CTAB-protocol (see above). Six oligonucleotide primers were then designed (Oligo Primer Analysis Software, version 5.0) to amplify three (A,B,C) overlapping PCR-fragments, spanning the entire coding sequence of this cellulose synthase gene on BAC-clone K2A11 (see Figure 2).

Fragment A was amplified with primers

F_a (5'-TTAGCCATCCCAAGATTCT-3') - - (SEQ ID NO. 20) - - and
 R_a (5'-CTTCAAGGGGTCAACAGTA-3') - - (SEQ ID NO. 21) - - giving a
 2034 bp PCR-product (bases 13939-15972 on K2A11)

Fragment B

F_b (5'-TACCGAGCGTTTTTCCTAT-3') - - (SEQ ID NO. 22) - -
 R_b (5'-CCAGCACCTAAGTTTCACA-3') - - (SEQ ID NO. 23) - -
 2064 bp PCR-product (bases 12382-14445 on K2A11)

Fragment C

F_c (5'-GTTCAGTTCACCAAAGATT-3') - - (SEQ ID NO. 24) - -
 R_c (5'-TCATTCCGACCAAAGTT-3') - - (SEQ ID NO. 25) - -
 2395 bp PCR-product (bases 10620-13014 on K2A11)

11. The paragraph starting on the 3rd full paragraph of page 23 (line 18) and continuing to page 24 line 4 should read as follows:

The *ixr1-2* mutation was mapped with high resolution to a small region on the top arm of chromosome V as described in materials and methods. The mapping results indicated that the *ixr1* mutations mapped very near to a gene, *Ath-B*, encoding cellulose synthase. A cDNA clone for the *Ath-B* mRNA has previously been described by Arioli et al. (1998). The nucleotide-sequence of this clone and the deduced amino acid sequence were deposited in GenBank as accession number AF027174. Comparison of the sequence of the cDNA to the genomic sequence present on BAC clone K2A11 (GenBank accession number AB018111) indicates that the cDNA clone has 87 nucleotides at the 5' end that are not present in the genomic sequence. This extra sequence corresponds to a 59 nucleotide multiple cloning site (G GACTC GCGCGC CTGCAG GTCGAC ACTAGTGGATCC AAA GAATTC G CGGCCG C GTCGAC, - - (SEQ ID NO. 26) - - restriction enzymes sites are shown in *italics*) that was introduced during cloning of the cDNA and an additional 28 nucleotide fragment of DNA

(TACGGCTGCGAGAAGACGACAGAAGGGG) - - (SEQ ID NO. 27) - - that was also introduced at some stage during the cloning of the cDNA (see bottom insert in Figure 2). A search of GenBank indicated that this sequence is also found at the 5' ends of other cDNA clones; thus it is a common artifact in some libraries.

12. Please insert the paper copy of the Sequence Listing attached herewith at the end of the specification.
13. Amend Figure 2 by inserting (SEQ ID NO. 3) under the nucleic acid sequence shown in the Figure. See revised Fig. 2 attached herewith.